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Genetic Requirements of Phage λ Red-Mediated Gene Replacement in *Escherichia coli* K-12

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Recombination between short linear double-stranded DNA molecules and *Escherichia coli* chromosomes bearing the *red* genes of bacteriophage λ in place of *recBCD* was tested in strains bearing mutations in genes known to affect recombination in other cellular pathways. The linear DNA was a 4-kb fragment containing the *cat* gene, with flanking *lac* sequences, released from an infecting phage chromosome by restriction enzyme cleavage in the cell; formation of Lac$^-$ chloramphenicol-resistant bacterial progeny was measured. Recombinant formation was found to be reduced in *ruvAB* and *recQ* strains. In this genetic background, mutations in *recF*, *recO*, and *recR* had large effects on both cell viability and on recombination. In these cases, deletion of the *sulA* gene improved viability and strain stability, without improving recombination ability. Expression of a gene(s) from the *nin* region of phage λ partially complemented both the viability and recombination defects of the *recF*, *recO*, and *recR* mutants and the recombination defect of *ruvC* but not of *ruvAB* or *recQ* mutants.

Efficient recombination involving the *Escherichia coli* chromosome takes place only when the recombining partner DNA is large and contains Chi sites to activate the recombination-promoting activities of RecBCD (for a review, see reference 20). Short linear DNA molecules in the cell generally are destroyed by RecBCD. Recombination between short linear DNA molecules and the host chromosome at a frequency high enough to be of practical use in making gene replacements has been observed with *recD* and *recBC sbcBC* mutant strains (12, 24). Still-higher frequency recombination is seen with *E. coli* strains in which the *recBCD* gene cluster is replaced by the *red* genes (*gam*, *bet*, and *exo*) of phage λ (19).

In addition to proceeding at high efficiency, Red-mediated recombination between a short linear DNA molecule and a circular homologue may represent a simpler recombination pathway than any of the previously characterized pathways for conjugational or transductional recombination. These properties of efficiency and (relative) simplicity recommend the hybrid phage-bacterial recombination system for research on general recombination mechanisms. In previous studies, we have shown that such recombination events require the activities of RecA, Exo, and Bet, as well as double-strand breaks (22). Murphy (19) found that the frequency is decreased by mutation of *recA* and *recF* and increased by mutation of *recJ*. The frequency of Red-mediated recombination is also elevated in a *recG* mutant strain. In the case of an event involving the insertion of substantial nonhomology (as in gene replacement), recombination in the *recG* host is apparently constrained to proceed through a pathway requiring RuvC resolvase (23).

In this study, we examined the dependence of Red-mediated gene replacement on several additional known *E. coli* recombination genes. Functional complementation between some of these genes and other phage λ genes was tested as well.

Strains. λ *lac*::*cat819 nin5* has been described previously (23). Bacterial strains employed in this study are described in Table 1. A number of them contain in vitro-assembled substitutions in which most of the coding sequence of a gene is replaced with a genetic element conferring resistance to either tetracycline or kanamycin or else with a short synthetic sequence that preserves the original reading frame. Details of these constructions will be described elsewhere (K. C. Murphy, K. Campellone, and A. R. Poteete, unpublished data).

Some of the strains described in Table 1 contain an insertion in the *galK* gene of sequences from the *nin* region of the λ chromosome, fused to the promoter P_{tac}, along with a kanamycin resistance-conferring determinant derived from Tn*903*. The P_{tac}-*nin* fusion was constructed in four steps. (i) The *Eco*RI fragment of the chromosome of λ cI857 S7 bearing the *nin* region (bp 39168 to 44972) was ligated into the *Eco*RI site of pBR322, in the orientation in which the direction of transcription of the λ genes would be clockwise in the conventional map of pBR322. (ii) Sequences of the resulting plasmid between the *Pst*I and *Sma*I sites, containing the N-terminal half of *bla*, were replaced by sequences between the *Pst*I and *Pvu*II sites of ptac12 (1) containing P_{tac}, with an *Xho*I linker (CCC TCGAGGG) inserted between the *Pvu*II and *Sma*I ends. (iii) Sequences of the resulting plasmid, containing the transcriptional terminator t_{R2}, were deleted by digestion with *Xho*I and *Stu*I, filling in with DNA polymerase I large fragment, and ligation with *Bgl*II linkers (CAGATCTG). (iv) Sequences between the filled-in *Hin*dIII sites of the resulting plasmid were replaced with *Eco*RI linkers (GGAATTCC). An *Eco*RI fragment from the resulting plasmid, bearing the P_{tac}-*nin* fusion, was then ligated into the *Eco*RI site of a *galK* insertion vector. In the resulting plasmid, pTP878, the λ sequences are transcribed in the *galK* antisense direction. The *galK* insertion vector was constructed from pTP838 (Murphy et al., unpublished data) by digestion with *Apa*I and *Sac*I, blunting the ends, and ligating *Eco*RI linkers between the flanking *gal* and *kan* sequences. The *galK*::P_{tac}-*nin kan* insertion of pTP878 was crossed into TP507 and TP554 (Table 1) by electroporation with *Pvu*II-digested plasmid DNA, as described previously (19); kanamycin-resistant recombinants were selected.

The presence of Tn*10*, Tn*5*, and mini-Tn*10-9*(Kan) in various genes in strains described in Table 1 was confirmed by production of appropriate-size DNA products in PCR with

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TABLE 1. Bacterial strains used in this study[a]

Strain	Relevant genotype	Source, reference(s), or construction
AB1157	F$^-$ *thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 supE44 galK2* λ$^-$ *rac*$^-$ *hisG4 rfbD1 mgl-51 rpsL31 kdgK51 cyl-5 mtl-1 argE3 thi-1 qsr′*$^-$	2
AM207	*recR252*::Tn*10-9kan*	16
BT12	*recF400*::Tn*5*	29
CS85	*eda-51*::Tn*10 ruvC53*	27
JC12123	*recJ284*::Tn*10*	15
JC15329	Δ(*srl-recA*)*306*::Tn*10*	A. J. Clark
JC15716	*recO1504*::Tn*5*	25
KM32	(*recC-ptr-recB-recD*)Δ::P_{tac}*-gam-red-cat*	19, 23
MV2104	*lexA71*::Tn*5*	14
N2057	*ruvA60*::Tn*10*	27
SS185	*recJ284*::Tn*10*	15
STL1548	*recQ1802*::Tn*3*	21
TP507	*AB1157* (*recC-ptr-recB-recD*)Δ::P_{tac}*-gam-red-pae-cI822*	23
TP522	*recG258*::*kan*	23
TP523	*ruvC53 eda*::Tn*10*	23
TP524	*recJ284*::Tn*10*	TP507 × P1(JC12123)
TP527	Δ(*srl-recA*)*306*::Tn*10*	TP507 × P1(JC15329)
TP531	*recG258*::*kan ruvC53 eda*::Tn*10*	TP522 × P1(CS85)
TP532	*recG258*::*kan* Δ(*srl-recA*)*306*::Tn*10*	TP522 × P1(JC15329)
TP535	*recG258*::*kan recJ284*::Tn*10*	TP522 × P1(JC12123)
TP538	*recG6200*(substitution of *tet857* for *cdn4-691*)	TP507 × linear DNA fragment[b]
TP540	*ruvAB6203*(substitution of *tet857* for [*ruvAcdn4-ruvBcdn334*])	TP507 × linear DNA fragment[b]
TP541	*ruvAB6204*(substitution of *kan858* for [*ruvAcdn4-ruvBcdn334*])	TP507 × linear DNA fragment[b]
TP554	*recG6202*(Δ*cdn4-691*)	TP538 × linear DNA fragment[b]
TP555	*recG6202 ruvC53 eda*::Tn*10*	TP554 × P1(CS85)
TP559	*recG6202 ruvAB6204*	TP554 × P1(TP541)
TP577	*recG6202 recF6206*(substitution of *tet857* for *cdn4-295*)	TP554 × linear DNA fragment[b]
TP590	*recJ284*::Tn*10*	TP507 × P1(SS185)
TP595	*recG6202 recJ284*::Tn*10*	TP554 × P1(SS185)
TP605	*sulA6209*(substitution of *tet857* for *cdn9-153*)	KM32 × linear DNA fragment[b]
TP606	*sulA6209*	TP507 × P1(TP605)
TP607	*recG6202 sulA6209*	TP554 × P1(TP605)
TP608	*sulA6209 lexA71*::Tn*5*	TP606 × P1(MV2104)
TP609	*recG6202 sulA6209 lexA71*::Tn*5*	TP607 × P1(MV2104)
TP612	*galK*::P_{tac}*-nin-kan878*	TP507 × linear DNA fragment[c]
TP614	*sulA6209 recO1504*::Tn*5*	TP606 × P1(JC15716)
TP615	*sulA6209 recF400*::Tn*5*	TP606 × P1(BT12)
TP617	*recG6202 galK*::P_{tac}*-nin-kan878*	TP554 × linear DNA fragment[c]
TP618	*galK*::P_{tac}*-nin-kan878 recF6206*	TP612 × P1(TP577)
TP620	*recQ1802*::Tn*3*	TP507 × P1(STL1548)
TP621	*recG6202 recQ1802*::Tn*3*	TP554 × P1(STL1548)
TP625	*sulA6209 recR252*::Tn*10-9kan*	TP606 × P1(AM207)
TP626	*recG6202 sulA6209 recO1504*::Tn*5*	TP607 × P1(JC15716)
TP627	*recG6202 sulA6209 recR252*::Tn*10-9kan*	TP607 × P1(AM207)
TP628	*recG6202 sulA6209 recF400*::Tn*5*	TP607 × P1(BT12)
TP629	*recG6202 galK*::P_{tac}*-nin-kan878 recF6202*	TP617 × P1(TP577)
TP630	*galK*::P_{tac}*-nin-kan878 ruvC53 eda*::Tn*10*	TP612 × P1(CS85)
TP631	*galK*::P_{tac}*-nin-kan878 ruvAB6203*	TP612 × P1(TP540)
TP632	*recG6202 galK*::P_{tac}*-nin-kan878 ruvC53 eda*::Tn*10*	TP617 × P1(CS85)
TP633	*recG6202 galK*::P_{tac}*-nin-kan878 ruvAB6203*	TP617 × P1(TP540)
TP634	*galK*::P_{tac}*-nin-kan878* Δ(*srl-recA*)*306*::Tn*10*	TP612 × P1(JC15329)
TP635	*recG6202 galK*::P_{tac}*-nin-kan878* Δ(*srl-recA*)*306*::Tn*10*	TP617 × P1(JC15329)
TP638	*recQ6216*(substitution of *tet857* for *cdn19-606*)	TP507 × linear DNA fragment[b]
TP639	*recG6202 recQ6216*	TP554 × linear DNA fragment[b]
TP640	*galK*::P_{tac}*-nin-kan878 recQ6216*	TP612 × linear DNA fragment[b]
TP641	*galK*::P_{tac}*-nin-kan878 recO6218*(substitution of *tet857* for *cdn3-240*)	TP612 × linear DNA fragment[b]
TP642	*galK*::P_{tac}*-nin-kan878 recR6213*(substitution of *tet857* for *cdn4-182*)	TP612 × linear DNA fragment[b]
TP643	*recG6202 galK*::P_{tac}*-nin-kan878 recQ6216*	TP617 × linear DNA fragment[b]
TP644	*recG6202 galK*::P_{tac}*-nin-kan878 recO6218*	TP617 × linear DNA fragment[b]
TP645	*recG6202 galK*::P_{tac}*-nin-kan878 recR6212*	TP617 × linear DNA fragment[b]

[a] Strains TP522 through TP645, except for TP605, were all constructed in the TP507 background and bear the substitution (*recC-ptr-recB-recD*)Δ::P_{tac}*-gam-red-pae-cI822*; they are presumed to bear all the other genetic markers of AB1157 as well, but these were not tested.

[b] Construction of *recF*, *recG*, *recO*, *recQ*, *recR*, *ruvAB*, and *sulA* substitution alleles will be described elsewhere (Murphy et al., unpublished data).

[c] Details of the construction are given in the text.

transposon- and chromosomal gene-specific primers. Cells from liquid cultures were pelleted, resuspended in water, and used directly as template. Primer tn5out (CCATGTTAGGA GGTCACATGGAAG) directs DNA synthesis from both ends of Tn*5* outward; primer mtn10 (GATCATATGACAAGATG TGTATCCACCTT) does the same for Tn*10* and mini-Tn*10*. Primers used for specific genes were as follows: edamU (CG CGGCCGGTATTCAGATTAACTG), recfU (ATCATCGA GCTCGAGATGGAAATGGTGGCACGTGTT), recfD (TC ATCAGAGCTCCGATTTCACCTCAGAAGAAACCAG), recjU (ATCATCGAGCTCAATTGACGTGTTGTTTCCCA GCCA), recjD (ATCATCGAGCTCTCCATCGCCTGTTTC TCGGCATTT), recoU (ATCATCGAGCTCCGCCGAACA GGCGTTGAAAAAACT), recoD (TCATCAGAGCTCGCT TTTGCTGCGGCTTCTTTCACA), recrU (ATCATCGAGC TCAAAGACTGGCTTCGGTCACCGAT), and recrD (TCA TCAGAGCTCCTGGTGTACTCCTGCTTACCTTCA).

Methods. Crosses between λ *lac*::*cat819 nin5* and bacterial strains were carried out as described previously (23). UV sensitivity was measured by plating bacteria on Luria-Bertani (LB) agar, exposing the plates to variable doses of UV (0, 10, 20, or 30 J/m^2, measured with a Spectronics DM-254N shortwave UV meter), and incubating them at 37°C in the dark. Fractional survival was determined by colony counts relative to the unexposed control.

Experimental system. Experiments to measure recombination between a linear DNA fragment and the *E. coli* chromosome employed bacterial strains bearing a (*recC-ptr-recB-recD*) Δ::P_{tac}-*gam-red-pae-cI* substitution. In these strains, the *E. coli* *recC*, *ptr*, *recB*, and *recD* genes are replaced by the recombination genes of phage λ, the *Pae*R7 restriction-modification system, and the phage λ *cI* gene. Log-phase cultures of these bacteria are infected with λ *lac*::*cat819 nin5*. The injected phage chromosome circularizes but does not proceed through the lytic or lysogenic cycle because of the cI repressor present in the cell. The chromosomally encoded *Pae*R7 restriction endonuclease cuts the (unmodified) phage DNA at two sites, releasing a 4-kb linear DNA fragment consisting of the *cat* gene flanked by 1.5-kb *lac* sequences. Recombination between this fragment and the chromosome frequently results in gene replacement; recombinants are detected as white colonies on LB agar plates supplemented with chloramphenicol, IPTG (isopropyl-β-D-thiogalactopyranoside), and X-Gal (23).

In addition to the white colonies formed by gene replacement, blue (Lac$^+$) chloramphenicol-resistant recombinants were formed as well. The numbers of Lac$^+$ recombinants were variable, amounting to 5 to 60% of the total chloramphenicol-resistant population, and were found in crosses with all mutant hosts (data not shown). In experiments involving electroporation with pure DNA fragment preparations, such Lac$^+$ recombinants were formed only when the DNA fragment bore one or two nonhomologous flanks (unpublished observations). The Lac$^+$ recombinants formed after infection with λ *lac*::*cat819 nin5*, therefore, presumably reflect recombination reactions that took place between the bacterial chromosome and phages that were cut only once by the *Pae*R7 endonuclease. The Lac$^+$ recombinants are the subject of ongoing investigation. Only Lac$^-$ recombinant production is considered below.

In the experiments reported below, defects in the production of recombinants are due to defects in the process of recombination, not in any of the steps involved in the delivery of the linear double-stranded DNA recombination substrate into the cell. All the strains that formed recombinants at reduced efficiency were found to plate *Pae*-modified λ *imm22* and λ *h80 imm22* at high efficiency (data not shown). This observation demonstrates that adsorption and injection of phage DNA are not impaired in these mutants. (It also demonstrates that these strains, many of which have poor viability, are as capable as the wild type of becoming infective centers.) In addition, all were found to plate unmodified λ *h80 imm22* with an efficiency of less than 0.001 (data not shown). This observation indicates that *Pae*R7 cutting is efficient in all of them.

TABLE 2. Effects of mutations on λ Red-mediated recombination and UV sensitivity in *E. coli* strains

Strain no.		Relevant genotype[a]	*recG*$^+$		*recG*	
recG$^+$	*recG*		Rec[b]	UV[c]	Rec[b]	UV[c]
507	554	Wild type	1.00 ± 0.18	R	1.00 ± 0.13	S
527	532	*recA*Δ	0.01 ± 0.01	S	0.01 ± 0.01	S
590	535	*recJ*	2.89 ± 0.93	S	0.10 ± 0.04	S
620	621	*recQ*	0.22 ± 0.02	I	0.22 ± 0.03	S
638	639	*recQ*Δ	0.05 ± 0.03	I	0.04 ± 0.02	S
540	559	*ruvAB*Δ	0.41 ± 0.07	S	0.27 ± 0.20	S
523	555	*ruvC*	0.26 ± 0.07	S	0.10 ± 0.03	S
606	607	*sulA*Δ	1.00 ± 0.31	R	1.00 ± 0.15	S
608	609	*sulA*Δ *lexA*	0.47 ± 0.05	R	0.35 ± 0.16	S
615	628	*sulA*Δ *recF*	0.10 ± 0.07	S	0.02 ± 0.02	S
614	626	*sulA*Δ *recO*	0.06 ± 0.01	S	0.01 ± 0.01	S
625	627	*sulA*Δ *recR*	0.02 ± 0.01	S	0.02 ± 0.01	S
612	617	*nin*$^+$	1.00 ± 0.31	R	1.00 ± 0.24	S
634	635	*nin*$^+$ *recA*Δ	0.02 ± 0.00	S	0.03 ± 0.01	S
618	629	*nin*$^+$ *recF*Δ	0.06 ± 0.03	S	0.71 ± 0.42	S
641	644	*nin*$^+$ *recO*Δ	0.21 ± 0.16	S	0.11 ± 0.06	S
642	645	*nin*$^+$ *recR*Δ	0.09 ± 0.05	S	0.19 ± 0.05	S
640	643	*nin*$^+$ *recQ*Δ	0.04 ± 0.02	I	0.04 ± 0.02	S
631	633	*nin*$^+$ *ruvAB*Δ	0.04 ± 0.01	S	0.05 ± 0.01	S
630	632	*nin*$^+$ *ruvC*	0.32 ± 0.09	S	0.94 ± 0.08	S

[a] All strains bear the *recBCD*Δ::P_{tac}-*gam-red-pae-cI822* substitution. Those listed under the columns headed by *recG* additionally bear an in-frame deletion of all but the first three and last three codons of *recG*.

[b] Normalized recombination frequency. Cultures were infected with λ *lac*::*cat819 nin5* at a multiplicity of 10. Following aeration for 1 h at 37°C, cells were plated on LB agar with or without chloramphenicol, IPTG, and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside. Averages and standard errors of the ratios of recombinants (white colonies) to total viable titers were determined from 2 to 20 measurements. These numbers were then divided by the frequencies of the parent strains listed at the heads of the strain groups (entries represented as 1.00 in the table). The actual recombination frequencies for these parent strains were as follows: TP507, 1.48%; TP606, 1.49%; TP612, 1.85%; TP554, 5.58%; TP607, 4.95%; and TP617, 3.91%.

[c] Sensitivity to UV was determined by survival of exposure to a dose of 10 J/m^2. R, resistant (more than 10% survivors); S, sensitive (less than 1%); I, intermediate (1 to 10%).

Dependence on recombination genes. Mutant alleles of genes *recA*, *recF*, *recJ*, *recO*, *recQ*, *recR*, *ruvA* and *ruvB*, and *ruvC* were introduced into TP507 (AB1157 *recBCD*Δ::P_{tac}-*gam-red-pae-cI822* [23]) and a derivative, TP554, in which the *recG* gene had been deleted (Table 1). Measurements of the recombination proficiencies of most of these strains are indicated in Table 2.

As reported previously, or as expected from the results of similar experiments, recombination in the *recG*$^+$ background was greatly decreased by mutation of *recA*, mildly decreased by mutation of *ruvC*, and stimulated by mutation of *recJ* (19, 23). Loss of *ruvAB* additionally caused a slight decrease in recombination rate. The *recQ1802*::Tn*3* allele reduced recombination approximately 5-fold, while a deletion-substitution allele reduced it approximately 20-fold.

In the *recG*Δ background, the recombination rate was elevated and it was reduced more by mutation of *ruvC* than it was in the *recG*$^+$ strains, as previously reported (23). In the *recG*Δ background, loss of *ruvAB* function led to a slightly greater loss

of recombination proficiency than in the *recG*$^+$ strain, but it is not clear that the difference is significant. As in the *recG*$^+$ strain, recombination was strongly dependent upon *recA*, reduced 5-fold by *recQ*::Tn*3* and 20-fold by *recQ*Δ. In comparing the *recG*$^+$ and *recG*Δ strains, the biggest difference in the dependence of recombination on specific *E. coli* genes was seen in the case of *recJ*. Loss of *recJ* function, while increasing recombination in the *recG*$^+$ strain, decreased it 10-fold in the *recG*Δ strain. While this observation might seem to indicate an interesting mechanistic relationship between RecG and RecJ proteins, it is unclear that the effect of the double mutant is specific. The *recG*Δ *recJ* strain is only marginally viable (data not shown), and its recombination deficiency might be secondary to other defects.

The *recF*, *recO*, and *recR* mutants in both *recG*$^+$ and *recG*Δ backgrounds exhibited even lower viability than did the *recG*Δ *recJ* strain, on the order of 1 CFU per 1,000 countable cells in log-phase culture (data not shown). Cultures of these mutants exhibited low, but highly variable, rates of recombination, possibly reflecting the outgrowth of revertants or pseudorevertants. We found that the viability of *recF*, *recO*, and *recR* mutants could be improved by deletion of *sulA* (an SOS-inducible gene, formerly known as *sfiA*, whose product is a cell division inhibitor; see reference 7) and so conducted tests in this background (Table 2). The *sulA* mutation itself has no effect on recombination frequency. Mutation of *recF*, *recO*, or *recR* substantially decreased recombination in both the *sulA*Δ *recG*$^+$ and *sulA*Δ *recG*Δ backgrounds.

We used the *sulA*Δ strain to test whether induction of the SOS regulon would increase Red-mediated recombination activity by increasing the intracellular levels of recombination functions. Introduction of the *lexA71*::Tn*5* mutation, however, slightly reduced recombination, in both the *recG*$^+$ and *recG*Δ backgrounds (Table 2).

The sensitivity of the bacterial strains to UV radiation was tested. Results are shown in Table 2. Mutations in all of the recombination genes tested significantly increased UV sensitivity, whether they decreased or increased the efficiency of recombination.

Proteins encoded by the recombination genes tested in this study have been extensively characterized. The RecF, RecO, and RecR proteins of *E. coli* form a complex that is thought to function in recombination by modulating the DNA binding of RecA (10, 32, 33). RecQ protein is a helicase which can cooperate with RecA and SSB proteins to initiate recombination-like events in vitro (9, 30). The RuvA and RuvB proteins form a complex that catalyzes the branch migration of crossover structures in DNA (17, 18). The complex of RuvAB with DNA is thought to direct the action of RuvC, which resolves Holliday junctions by making strand-specific cuts (31).

Complementation by λ functions. Two genes in the *nin* region of phage λ, *orf* and *rap*, function in homologous recombination. The *orf* gene can substitute for *recF*, *recO*, and *recR* in phage recombination mediated by the host system in *recBC sbcBC* cells in the absence of the λ Red system (26). The *rap* gene encodes an endonuclease which specifically cleaves branched DNA structures, including Holliday junctions, that are thought to be generated during recombination and which hypothetically might substitute for RuvC (11, 28). We tested whether λ *nin* genes could functionally replace any of the *E. coli* genes needed for Red-mediated gene replacement, by inserting a P_{tac} fusion of *orf* and other λ genes downstream of it into the *galK* gene. Details of the construction are given above.

Complementation activity was seen with both *recG*$^+$ and *recG*Δ backgrounds but more strongly in the *recG*Δ strain. As shown in Table 2, in the *nin*$^+$ *recG*Δ background, loss of *recF*, *recO*, or *recR* function reduced recombination, but less than in the *recG*Δ strain lacking *nin* functions. The smaller effects of *recF*, *recO*, and *recR* mutations in this background occurred in spite of the fact that the alleles used in this case were deletions. Partial complementation of the defects of *recF*, *recO*, and *recR* mutants by *nin* was also evident in the viability of these mutant strains, which was better than that of their *nin*-less counterparts (data not shown). The *nin* genes also were seen to compensate for loss of *ruvC* in gene replacement recombination. In

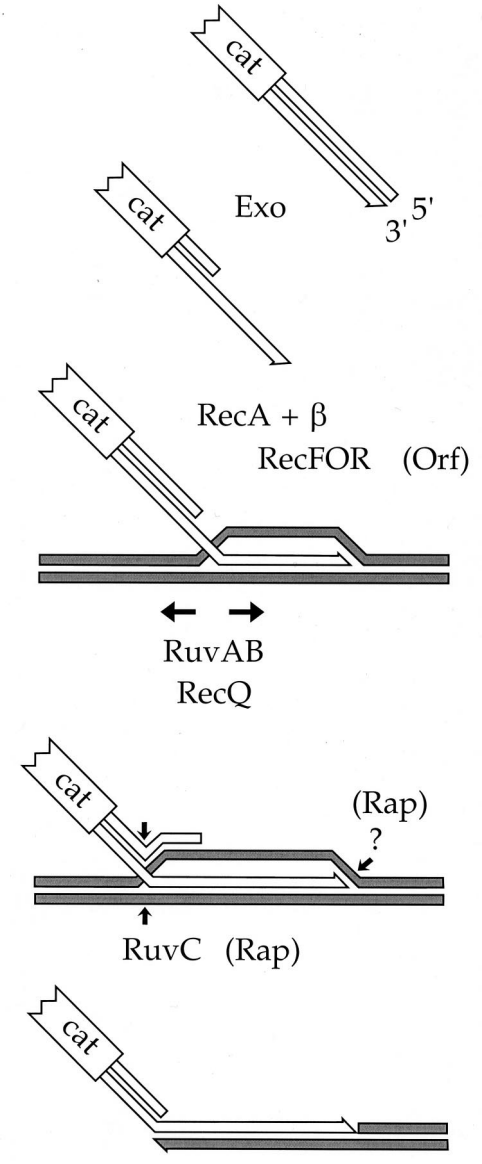

FIG. 1. Possible molecular events in Red-mediated replacement of *lac* with *cat* in the chromosome of *E. coli recG*Δ. It is assumed that such events would have to take place on both sides of the *cat* gene; for clarity, only one side is shown. Recombination is initiated by a double-strand break. λ exonuclease processively digests the 5′-ended strand, leaving a 3′ single-stranded tail. RecA, in conjunction with the λ beta protein, and either host-encoded RecFOR or λ Orf, mediates invasion of the 3′-ended strand into an unbroken homologous duplex. Once formed, the crossed strands are subject to RuvAB and/or RecQ helicase-driven branch migration, resulting in a Holliday junction, which can be resolved by either RuvC or Rap into a recombinant molecule. No role for DNA synthesis is involved in this particular scheme, but of course the invading 3′ end could serve as a primer for repair synthesis.

contrast, *nin* did nothing to remedy the recombination defects of *recA*, *recQ*, or *ruvAB* mutants. The *nin* genes did not compensate for loss of any of the recombination genes in providing resistance to the lethal effects of UV radiation (Table 2).

Red mechanism. A hypothetical mechanism for Red-mediated recombination leading to gene replacement in *E. coli recG*Δ, based on an earlier scheme (23), on results shown in Table 2, and on the research on recombination proteins cited above, is shown in Fig. 1. Results presented above do not distinguish which λ proteins encoded by *nin* genes contribute to complementation of the recombination defects of mutant strains lacking RecFOR or RuvC. However, research by others on *orf* and *rap* strongly suggests that these are the relevant functions (11, 26, 28). The mechanism shown in Fig. 1 accounts for the main pathway to recombinant formation in a *recG* mutant cell. In a $recG^+$ cell, other pathways, frequently not leading to recombinant formation, are apparently more prevalent (23).

Relationship to other pathways. Results described above and in previous work (19, 23) indicate the importance of *recA*, *recF*, *recO*, *recR*, *recQ*, *ruvAB*, and *ruvC*, and the inhibitory influence of *recJ* and *recG*, in Red-mediated gene replacement in *E. coli* lacking RecBCD function. These observations distinguish the hybrid phage-bacterial recombination pathway from the classical RecF and RecE pathways for conjugational and transductional recombination, in that the latter are partially dependent upon both *recJ* and *recG* (for a review, see reference 13). However, strains of *E. coli* bearing chromosomal substitutions of the λ *red* genes for the *recC-ptr-recB-recD* cluster are particularly analogous to *recB recC sbcA* strains, in which RecBCD is functionally replaced by the induced recombination functions *recE* and *recT* of the cryptic lambdoid prophage Rac (3–6, 8). A more direct comparison would be needed to determine whether the Red pathway (with or without expression of other λ recombination genes) is mechanistically different from the RecE pathway.

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